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HIGHLIGHTED TOPIC | *Muscle Dysfunction in COPD*

The mechanisms of cachexia underlying muscle dysfunction in COPD

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Remels AH, Gosker HR, Langen RC, Schols AM. The mechanisms of cachexia underlying muscle dysfunction in COPD. *J Appl Physiol* 114: 1253–1262, 2013. First published September 27, 2012; doi:10.1152/japplphysiol.00790.2012.—Pulmonary cachexia is a prevalent, debilitating, and well-recognized feature of COPD associated with increased mortality and loss of peripheral and respiratory muscle function. The exact cause and underlying mechanisms of cachexia in COPD are still poorly understood. Increasing evidence, however, shows that pathological changes in intracellular mechanisms of muscle mass maintenance (i.e., protein turnover and myonuclear turnover) are likely involved. Potential factors triggering alterations in these mechanisms in COPD include oxidative stress, myostatin, and inflammation. In addition to muscle wasting, peripheral muscle in COPD is characterized by a fiber-type shift toward a more type II, glycolytic phenotype and an impaired oxidative capacity (collectively referred to as an impaired oxidative phenotype). Atrophied diaphragm muscle in COPD, however, displays an enhanced oxidative phenotype. Interestingly, intrinsic abnormalities in (lower limb) peripheral muscle seem more pronounced in either cachectic patients or weight loss-susceptible emphysema patients, suggesting that muscle wasting and intrinsic changes in peripheral muscle's oxidative phenotype are somehow intertwined. In this manuscript, we will review alterations in mechanisms of muscle mass maintenance in COPD and discuss the involvement of oxidative stress, inflammation, and myostatin as potential triggers of cachexia. Moreover, we postulate that an impaired muscle oxidative phenotype in COPD can accelerate the process of cachexia, as it renders muscle in COPD less energy efficient, thereby contributing to an energy deficit and weight loss when not dietary compensated. Furthermore, loss of peripheral muscle oxidative phenotype may increase the muscle's susceptibility to inflammation- and oxidative stress-induced muscle damage and wasting.

COPD; skeletal muscle; cachexia; energy metabolism; muscle mass regulation

CACHEXIA IS A COMPLEX, DEBILITATING metabolic syndrome associated with an underlying chronic illness and is characterized by involuntary and progressive loss of skeletal muscle mass with variable loss of fat mass (33). Chronic obstructive pulmonary disease (COPD) is a lung disorder with progressive airflow obstruction resulting from inflammation and remodeling of the airways, which often includes development of emphysema. The prevalence of cachexia is high in COPD (20–40% depending on definition and disease stage) and appears more prevalent in the emphysematous phenotype (53, 107). Pulmonary cachexia increases mortality and is associated with poor quality of life and loss of peripheral and respiratory muscle function (30, 90). Interestingly, studies in food-restricted animals (52) and in anorexia nervosa patients (16) show that weight loss per se results in an emphysema-like pulmonary phenotype highlighting the complex interrelation between cachexia and pulmonary function.

The clinical diagnosis of cachexia is traditionally based on determination of body weight or body mass index (BMI). However, this approach warrants caution. First, muscle wasting is not only present in underweight COPD patients, but may also present itself in normal-weight patients with relative preservation of whole body fat mass or specific accumulation of visceral fat (37, 103). Therefore, without additional assessment of fat-free mass (FFM), "hidden" loss of muscle mass may remain undetected. Moreover, FFM index (FFMI) is an independent determinant of mortality in COPD and, compared with BMI, is a better predictor of muscle weakness and impaired exercise capacity (90, 103). Additionally, cachexia is a process and the time course of weight loss is often not reported in COPD literature. In this review, we will refer to patients as underweight (low BMI only), muscle-atrophied (low FFMI and normal BMI), or cachectic (low FFMI and low BMI) based on reported body composition (90).

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Muscle wasting in COPD progresses gradually, but likely is accelerated during acute disease exacerbations. Weight loss prior to as well as during hospitalization for a COPD exacerbation is associated with an increased risk for impaired recovery and hospital readmission (74). Furthermore, loss of FFM and muscle strength is more frequent and more severe in repeatedly exacerbating patients (4, 49). A more gradual loss of muscle and fat mass, on the other hand, appears more prevalent in advanced disease and in the emphysematous phenotype (32, 53) where cachexia has been associated with increased severity of emphysema (84).

The observation that FFM wasting can occur with or without decreases in BMI suggests that different pathophysiological mechanisms (protein metabolism *vs.* energy metabolism) may affect muscle function in pulmonary cachexia. Specifically, alterations in energy metabolism in COPD appear different from other chronic wasting diseases and we postulate that these abnormalities may even accelerate cachexia in COPD. In addition to peripheral muscle wasting, pulmonary cachexia is also associated with diaphragm atrophy and weakness, but intrinsic alterations in energy metabolism in respiratory and peripheral muscles appear distinct.

MUSCLE MASS MAINTENANCE IN COPD

Muscle wasting in COPD is, at least partly, the result of a decrease in the size of individual muscle fibers (13, 40). Whereas peripheral (lower limb) muscle fiber atrophy in COPD seems selective for the glycolytic type II fibers (40), diaphragm atrophy is characterized by a more generalized reduction in the size of all fiber types (14). Interestingly, malnutrition or nutritional deprivation in animal models also result in significant diaphragm atrophy and loss of diaphragm contractile force (26, 39), indicating that malnutrition per se can lead to diaphragm atrophy.

Muscle mass is determined by the net balance of protein synthesis and protein breakdown as well as myonuclear loss and accretion (apoptosis vs. regeneration), as represented in Fig. 1.

Protein Turnover

In COPD, on a whole body level, increased protein turnover has been reported (29, 51). It is, however, currently unclear whether cachexia is associated with changes in muscle protein synthesis. While one group reported decreased muscle protein synthesis in underweight emphysema patients (68), others, at whole body level, showed no difference or even an increase in protein synthesis in cachectic patients compared with controls or non-cachectic patients (51, 87). No data exist on synthesis and degradation rates of muscle-specific proteins, albeit that (whole body) myofibrillar protein degradation was found to be increased in underweight patients compared with controls and normal-weight patients (87). Indirect evidence for increased muscle-protein degradation was shown in emphysematous and underweight COPD patients based on increased circulatory levels of methylhistidine, a product of muscle protein breakdown (99). Additionally, increased levels of pseudo-uridine (a proposed marker for cellular protein breakdown) in urine of COPD patients correlated inversely with FFM (10).

Protein Degradation Pathways

Although several proteolytic systems in skeletal muscle can degrade contractile proteins, the ubiquitin-proteasome system (UPS) is considered the main proteolytic system involved in muscle atrophy. The muscle-specific E3 ligases atrogin-1 and

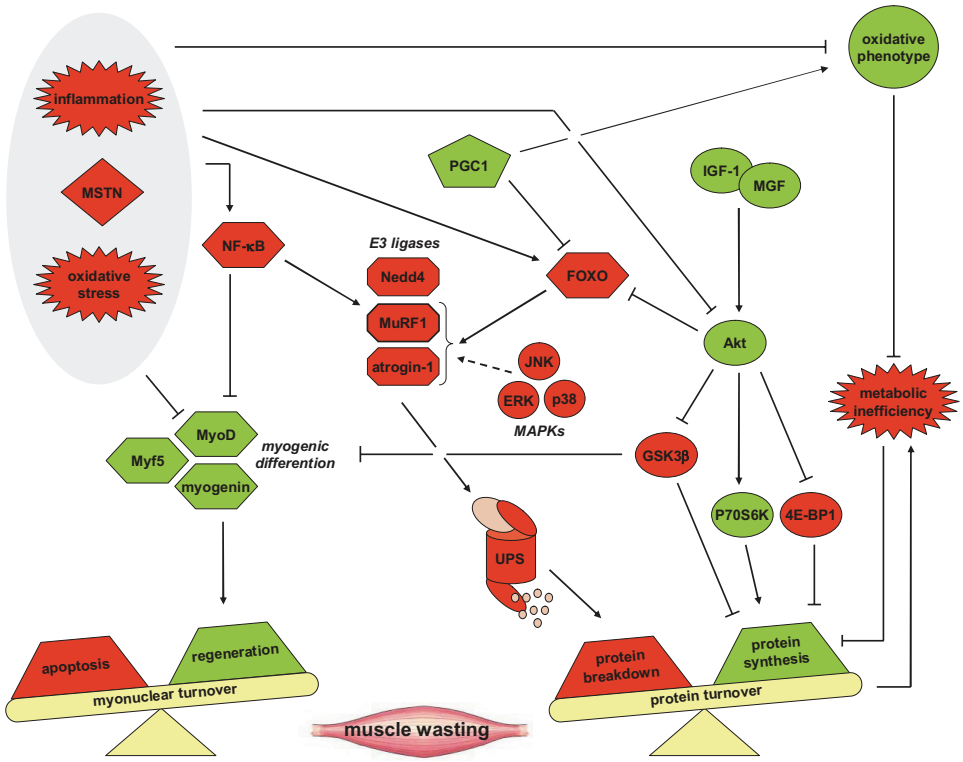

Fig. 1. Schematic representation of the triggers and underlying mechanism of cachexia in COPD. Myostatin (MSTN), peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1), Forkhead box O (FOXO), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), muscle atrophy F-box (Atrogin-1), muscle-specific ring finger 1 (MuRF1), Insulin-like growth factor-1 (IGF-1), mechanogrowth factor (MGF), serine/threonine protein kinase AKT (AKT), glycogen synthase kinase 3β (GSK3β), eIF4E-binding protein 1 (4E-BP1), ribosomal protein S6 kinase (P70S6K), ubiquitin-proteasome system (UPS), mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK).

muscle-specific RING finger protein 1 (MuRF1), regulated by fork-head transcription factors FOXO-1 and 3, are key players in UPS-dependent protein degradation (38).

Atrogin-1 mRNA and protein levels, not MuRF1, were found to be increased in peripheral muscle of muscle-atrophied COPD patients compared with controls (59, 73). In cachectic patients, atrogin-1 mRNA and protein as well as MuRF1 mRNA and total protein ubiquitination were increased compared with controls (27, 34). Moreover, muscle atrogin-1 protein content, not MuRF1 protein, was specifically increased in cachectic COPD patients compared with non-cachectic patients (105). In line with these observations, FOXO-1 and FOXO-3 mRNA levels were shown to be increased, compared with controls, in peripheral muscle of cachectic and muscle-atrophied COPD patients. In addition, muscle FOXO-1 nuclear content was found to be increased, compared with controls, in cachectic patients (21, 27, 34). A drawback for interpretation of data on FOXO, however, is that Doucet et al. (27) only reported nuclear, not total FOXO-1 protein levels, and did not investigate phosphorylation status of FOXO-1. In addition to increased atrogin mRNA and protein, muscle Nedd4 (another E3 ubiquitin-protein ligase) protein levels were also found increased in muscle-atrophied patients with severe COPD compared with controls (73).

Recently, increased atrogin-1 protein expression in muscle-atrophied COPD patients (FFMI: 17.5 $\pm$ 0.5 vs. 19.8 $\pm$ 0.5 in controls) was associated with increased mitogen-activated protein kinase (MAPK) activation (p38, ERK 1/2, and JNK) which inversely correlated with mid-thigh cross-sectional area (59). In contrast, compared with controls, others found no differences in MAPK activation in muscle-atrophied COPD patients (FFMI: 16.1 $\pm$ 2.2), although it must be noted that the control group had a relatively low FFMI (17.2 $\pm$ 2.2) as well, which may have masked potential differences (86). Additionally, Fermoselle et al. (34) found no differences in MAPK protein levels between controls, cachectic, and non-cachectic patients, which must, however, be interpreted with caution because phosphorylated MAPK protein levels would have been more informative in terms of activation of these pathways (34). Importantly, convincing evidence for a direct role of MAPK in the induction of E3 ligases in conditions of muscle atrophy is lacking. In line with well-described diaphragm atrophy in COPD, protein degradation seems enhanced in the diaphragm (e.g., increased proteasome activity and atrogin-1 mRNA expression) as recently reviewed by Caron et al. (13). Collectively, activation of the UPS has been consistently shown in peripheral (lower limb) muscle of both muscle-wasted patients and cachectic patients, whereas data on muscle UPS activation in muscle of normal-weight or underweight patients are lacking.

Protein Synthesis Pathways

The insulin-like growth factor-1 (IGF-1)-Akt signaling cascade is an essential pathway for muscle anabolism and hypertrophy. Circulating IGF-1 levels were unaltered in mild, moderate, and severe COPD compared with controls (72) and in cachectic vs. non-cachectic patients (22). However, muscle IGF-1 protein was decreased in cachectic COPD patients compared with non-cachectic patients (105), and serum and muscle IGF-1 levels were lower in acute exacerbating COPD patients compared with controls (19, 54).

Interestingly, in addition to increased expression of E3 ligases, increased activation of Akt was observed in peripheral muscle of cachectic patients vs. controls (27, 105). Downstream of Akt, the expression levels of 4E-BP1, P70S6K, and GSK-3β were increased specifically in cachectic patients compared with non-cachectic patients (27). The net result on protein synthesis remains difficult to interpret as these mediators have opposing effects on mRNA translation (see Fig. 1). Moreover, interpretation is also limited because the ratio of phosphorylated over total protein (which was lacking) is most informative in terms of pathway activation. Nevertheless, activation of muscle hypertrophic signaling pathways in the presence of cachexia could be interpreted as a futile attempt to restore muscle mass. In muscle-atrophied patients on the other hand, Akt phosphorylation was unaltered while muscle IGF-1 mRNA levels were increased compared with controls (60, 73). No data exist on protein synthesis pathways in COPD diaphragm. In summary, although circulating IGF-1 levels appear to be unaltered, downstream Akt signaling is activated compared with controls in lower limb muscle of cachectic patients but not in muscle-atrophied patients.

Myonuclear Turnover

The concept of a "myonuclear domain" (a given nucleus can only control a certain volume of cytoplasm) implies that a myofiber upon loss or gain of volume will have to adapt its nuclear content. Therefore, loss of muscle mass may not only involve changes in protein metabolism, but also involves changes in loss and accretion of myonuclei in muscle fibers. Loss of myonuclei is predominantly controlled by apoptotic events. Accretion of myonuclei is the result of fusion of mononuclear myoblasts with adjacent myofibers. These cells arise from locally residing "satellite cells" and subsequently differentiate under the influence of myogenic differentiation factors.

Apoptotic Mechanisms

Literature regarding muscle cell apoptosis in COPD is still controversial. Some groups reported increased muscle cell apoptosis (as reflected by DNA fragmentation) in underweight patients compared with controls and normal-weight patients (3, 7), whereas others showed no differences in active caspase-3 (as a marker for apoptosis) in muscle-atrophied patients compared with controls (42). Increased apoptosis has been observed in diaphragm of animal models of emphysema or pulmonary inflammation (23, 47) and recently also in COPD diaphragm (7).

Regeneration Pathways

Impaired muscle regeneration may contribute to muscle atrophy in COPD, as it inhibits myonuclear accretion of myofibers, limiting their capacity for (re)growth. In this context, several groups have assessed, in muscle of COPD patients, mRNA and protein expression levels of constituents of key pathways involved in muscle regeneration. Muscle mRNA levels of myogenic differentiation factors Myf5, and myogenin and MyoD protein levels were unaltered in muscle-atrophied COPD patients compared with controls (73). However, muscle

myogenin protein expression was lower in cachectic patients compared with controls, and MyoD protein was found significantly lower in cachectic vs. non-cachectic COPD patients (34, 105). In addition, patients undergoing an acute exacerbation displayed reduced muscular levels of MyoD protein compared with healthy controls (19). This data point toward decreased expression levels of myogenic differentiation factors, which are essential for muscle regenerative capacity, specifically in skeletal muscle of cachectic COPD patients. Satellite cell activation, increased MGF, and embryonic myosin heavy chain (MyHC) mRNA expression have been shown in diaphragm of COPD patients, suggesting an ongoing regenerative process (69). In addition, animal models of emphysema revealed a decreased diaphragmatic content of MyoD protein (23).

TRIGGERS OF CACHEXIA IN COPD

An inactive lifestyle is a common characteristic of COPD and may have profound effects on peripheral skeletal muscle function. However, because disuse preferentially affects type I fibers, type II fiber atrophy in COPD reflects pathological mechanisms additional to physical inactivity, of which oxidative/nitrosative stress, myostatin, and inflammation have been investigated as putative catabolic triggers (Fig. 1).

Oxidative/Nitrosative Stress

Reactive oxygen species (ROS) result from mitochondrial O_2 consumption or from other oxidant producing systems in the muscle as, e.g., the xanthine oxidase system. In addition to their direct oxidizing effects, ROS can give rise to reactive nitrogen species (RNS) by interacting with nitric oxide (NO). Oxidative/nitrosative stress can induce cell damage and cell death, impair myogenic differentiation (57), activate the UPS, and block protein synthesis (75), all of which may contribute to muscle atrophy.

An imbalance between the production of reactive species and antioxidant capacity leads to oxidative/nitrosative stress. In this regard, mitochondrial H_2O_2 production at rest (76) and exercise-induced xanthine oxidase activity were increased significantly in muscle of normal-weight patients compared with healthy subjects (24). In addition, basal antioxidant levels were reduced in peripheral musculature and circulation of emphysematous COPD patients compared with controls and in cachectic patients vs. non-cachectic patients (31, 101). Also, exercise-induced increases in antioxidant enzymes were attenuated or inhibited in underweight and normal-weight patients compared with controls (77, 78). Collectively, this results in increased systemic (circulation) and local (muscle) oxidative/nitrosative stress in COPD at rest and after acute exercise (34, 111).

Systemic and local oxidative stress in COPD patients correlated negatively with FFM and muscle strength, suggesting a link between oxidative stress and loss of muscle mass (8, 100). Furthermore, cachectic COPD patients exhibited greater muscle and systemic oxidative/nitrosative stress at rest and after exercise compared with non-cachectic patients and controls (8, 101). In line with these data, mitochondrial ROS production was found significantly greater in cachectic patients compared with non-cachectic patients and was associated with enhanced UPS activity and protein ubiquitination (34). In contrast, Fermoselle et al. (34) showed increased oxidative stress markers regardless of body composition, whereas only cachectic patients exhibited additional activation of proteolytic systems and atrophy of type II muscle fibers. Similar to peripheral muscle, increased oxidative stress is observed in COPD diaphragm, which may contribute to atrophy (6, 64).

Myostatin

Myostatin can induce muscle atrophy through inhibition of myoblast proliferation (by inhibiting MyoD), increasing UPS activity, and inhibiting anabolic signaling through the IGF/Akt pathway.

Myostatin mRNA expression in (lower limb) muscle was found increased in muscle-atrophied COPD patients compared with controls (73) and inversely correlated with quadriceps strength and endurance in patients (108). Myostatin protein levels in muscle, however, were unchanged in cachectic vs. non-cachectic patients (105). As myostatin produced in muscle is processed and excreted from the tissue (48), serum myostatin protein may serve as a biomarker. Indeed, circulatory myostatin levels were elevated in COPD patients compared with controls and correlated inversely with muscle mass (in men only) (50). It is unknown if and by which exact mechanisms myostatin might contribute to muscle atrophy in COPD. However, Plant et al. (73) associated increased muscle myostatin mRNA levels in muscle-atrophied patients compared with controls with increased UPS activity. Summarized, the available data suggest that muscle myostatin expression is elevated in muscle-atrophied and cachectic COPD patients compared with controls.

An overview of described alterations in protein degradation/synthesis and regeneration/apoptosis pathways in muscle of COPD patients is provided in Table 1. Comparisons between normal- and underweight patients and controls are excluded because of a lack of data in literature.

Inflammation

Inflammatory mediators have long been implicated in muscle atrophy and cachexia (81, 92). In particular, tumor necrosis factor-α (TNF-α) was originally designated "cachectin" in recognition of its catabolic actions. Historically, Beutler and Cerami (13a) were the first to describe a link between TNF-α and a cachectic phenotype characterized by anorexia and weakness. Follow-up studies demonstrated that experimental animals lose weight and muscle mass and display muscle weakness when treated with TNF-α or exposed to interventions that elevate endogenous TNF-α (e.g., sepsis or tumor implantation) (35, 66). Both acute and chronic models of inflammation result in muscle atrophy (35, 56). Currently, a causal role for inflammatory cytokines, especially TNF-α, has been established in several in vivo and in vitro models of inflammation-induced muscle atrophy (92, 95). Mechanisms by which TNF-α may mediate muscle atrophy include UPS activation and enhanced proteolysis, but also inhibition of protein synthesis and blockade of myogenic differentiation and muscle regenerative potential (56, 81). In particular, the inflammatory signaling pathway nuclear factor κB (NF-κB) has been causally implicated in (inflammation-induced) loss of muscle mass in experimental models in vivo and in vitro. NF-κB activation stimulates the proteasomal machinery and leads to significant loss of muscle

Table 1. *Protein synthesis/degradation and apoptosis/regeneration pathways in skeletal muscle of COPD patients*

	COPD Muscle Atrophy versus Healthy Controls	Cachectic COPD versus Healthy Controls	Cachectic COPD versus Noncachectic COPD
	Protein Degradation		
MuRF1 mRNA	=[59,73]	↑[27]	ND
MuRF1 protein	=[59,73]	=[34]	=[105]
Atrogin mRNA	↑[59,73]	↑[27]	ND
Atrogin protein	↑[59]	↑[27,34]	↑[105]
Nedd4 protein	↑[73]	ND	ND
FoxO mRNA	↑[21]	↑[27]	ND
FoxO protein	ND	↑[27,34]	ND
Poly-ubiquitinated protein	↑[59]	↑[34]	ND
	Protein Synthesis		
Muscle IGF-1 mRNA	↑[60]	ND	ND
Muscle IGF-1 protein	ND	ND	↓[105]
Circulating IGF-1	ND	=[27]	=[22]
P-Akt/Total Akt	=[73]	↑[27]	↑[105]
	Apoptosis		
TUNEL	=[42]	ND	ND
Caspase-3	=[42]	ND	ND
	Regeneration		
Myf 5mRNA	=[73]	ND	ND
Myogenin mRNA	=[73]	ND	ND
Myogenin protein	ND	↓[34]	ND
MyoD mRNA	ND	ND	ND
MyoD protein	=[73]	ND	↓[105]
	Myostatin		
Muscle myostatin mRNA	↑[73]	ND	ND
Muscle myostatin protein	ND	↑[34]	=[105]
	Oxidative Stress		
Muscle protein oxidation	↑[59]	↑[34]	↑[101,8]
Muscle anti-oxidants	ND	↑[34]	↑[34,8] ↓[31]
	Inflammation		
Muscle TNF-α	=[21] ↑[67]	=[34] ↓[9] ↑[82]	=[105] ↑[82]
Muscle NF-κB activity	↑[73]	↑[34]	↑[105]

MuRF1, muscle-specific RING finger protein 1; Nedd4, neuronal precursor cell-expressed developmentally downregulated 4; FoxO, fork-head transcription factor; IGF-1, insulin growth factor 1; Akt, protein kinase B; TUNEL, terminal uridine nick-end labeling; Myf5, myogenic factor 5; MyoD, myogenic differentiation antigen; TNF-α, tumor necrosis factor α; NF-κB, nuclear factor κB; ND, not determined. Numbers indicate relevant references.

mass, whereas NF-κB inhibition protects against the development of muscle atrophy in several experimental models (11, 62). Interestingly, NF-κB activation is required for the transition of systemic inflammation to muscle atrophy in a model of pulmonary inflammation (55).

COPD is often associated with a chronic low-grade inflammatory state with increased circulating levels of TNF-α, soluble TNF-α receptors, C-reactive protein (CRP), and interleukin (IL)-1β and IL-6 being reported (70). Although systemic inflammation is not present in all patients, a significant subset of patients displays persistent systemic inflammation that relates to increased mortality (1). In addition, acute exacerbations result in enhanced pulmonary and systemic inflammation and the presence of systemic inflammation is associated with an increased exacerbation frequency (1, 94). The exact relationship of systemic inflammation with the process of muscle wasting in COPD, however, is unclear because of a lack of longitudinal data. In addition, the presence of local inflammation in skeletal muscle of COPD patients is controversial. Some studies showed increased levels of TNF-α in the peripheral muscle of selected COPD patients (67, 82), whereas others failed to reproduce these results (9, 19). To date, these apparent discrepancies remain unexplained but differences in study population characteristics in terms of disease severity and body composition may play a role. However, regardless of TNF-α expression in muscle, data regarding activation of the nuclear factor κB (NF-κB) pathway in COPD skeletal muscle does suggest an increased inflammatory state of peripheral muscle tissue in this disorder. Indeed, increased muscle NF-κB activation was shown in severely underweight patients and in cachectic patients and muscle-atrophied patients compared with controls (2, 34, 73) but also in cachectic patients compared with non-cachectic patients (105). Recently, increased NF-κB activation in quadriceps muscle of cachectic COPD patients compared with controls was also found to correlate with total muscle protein ubiquitination (34). These authors, however, only assessed total, not nuclear, protein levels of RelA in muscle (transcriptional active NF-κB subunit), which is not informative with regard to NF-κB activation. Collectively, although scarcely available, data do point toward increased NF-κB activation in peripheral skeletal muscle of COPD patients.

Some groups have investigated inflammatory cell infiltration in muscle tissue of COPD patients as a potential source for inflammatory mediators affecting the muscle. Compared with controls, however, no differences were found in muscle inflammatory cell infiltration in normal-weight COPD patients or in patients with evident muscle atrophy (42, 72). In contrast, Barreiro et al. (7) did show increased numbers of inflammatory cells in muscle tissue from normal-weight COPD patients compared with controls. These authors, however, reported very low counts for inflammatory cells in muscle of both patients and controls which could underlie apparent discrepancies between studies.

In COPD, elevated circulating levels of TNF-α and its soluble receptors have been associated with acute weight loss (25) and a reduced lean mass (28). Also, systemic inflammation was more pronounced in cachectic patients compared with non-cachectic patients (101) and patients who fail to gain weight in response to nutritional support are characterized by high circulating levels of soluble TNF-α receptors (17). However, no reports are available addressing via which mechanisms (increased degradation, impaired protein synthesis, or impairment of regeneration) chronic inflammation and NF-κB activation contribute to muscle wasting in COPD.

MUSCLE METABOLISM IN CACHEXIA IN COPD

Although loss of muscle mass results in decreased strength, intrinsic muscle metabolic abnormalities impair muscle endurance, thereby further contributing to muscle dysfunction in COPD. In peripheral muscle, a fiber-type shift (I-II) toward a higher proportion of glycolytic fibers in COPD has been associated with disease severity (44) and appeared aggravated in emphysematous patients (43, 89).

Oxidative Metabolism

In addition to a fiber-type shift, COPD peripheral muscle has a decreased content of high-energy phosphates (ATP and phosphocreatine) at rest with a faster drop and slower recovery upon exercise, independent of nutritional status, indicative of an impaired oxidative metabolism (46, 91). Indeed, activity levels of mitochondrial oxidative enzymes (Krebs cycle and β-oxidation) were reduced in peripheral musculature of stable COPD patients, with decreases tending to be more severe in the emphysematous patient (20, 42, 63). In concordance, decreased numbers of mitochondria, but normal intrinsic mitochondrial function, were shown in lower limb muscle of normal-weight COPD patients compared with controls (41, 76). Intriguingly, mitochondrial function was particularly impaired in cachectic patients compared with non-cachectic patients (79). Moreover, cachectic patients in this study had a lower diffusing capacity of the lung for carbon monoxide (suggestive of emphysema) and a reduced endurance capacity compared with non-cachectic patients even when corrected for FFM (79). Collectively, muscle oxidative capacity and fiber-type composition will be referred to as oxidative phenotype. Peroxisome proliferator-activated receptors (PPAR)-α and -δ and their coactivator PPAR coactivator-1α (PGC-1α) together with mitochondrial transcription factor-A promote mitochondrial biogenesis and a slow fiber-type muscle phenotype. In line with their known function, protein and mRNA levels of these constituents were decreased in COPD muscle compared with controls, which was particularly pronounced in cachectic patients (82, 83). These data show that muscle oxidative phenotype and its molecular regulation by the PGC/PPAR signaling pathway is impaired in COPD, which is most pronounced in cachectic patients.

Glycolytic Metabolism

In addition to an impaired oxidative phenotype, increased glycolytic metabolism has been observed in peripheral muscle of COPD patients at rest and after exercise (45, 46, 88). Also, exercise-induced lactate production (i.e., enhanced anaerobic glycolysis) occurred earlier in cachectic patients compared with non-cachectic patients (79). In line with these studies, whole body glucose production was increased in normal-weight COPD patients upon exercise, whereas underweight patients already displayed increased glucose production at rest (36). Additionally, normal-weight COPD patients displayed a faster drop in muscle PH in response to mild exercise, and glycogen utilization and lactate accumulation required a lower work load and time to occur in normal-weight patients compared with controls (88). Similar to impairments in muscle oxidative phenotype, enhancement of glycolytic metabolism is more pronounced in cachectic patients. In line with this notion, stimulation of mitochondrial oxidative metabolism attenuated exercise-induced lactate production in COPD, suggesting that impairments in muscle oxidative metabolism increase the muscle's reliance on glycolysis as a source of energy generation (12).

Unlike peripheral muscle, an increased proportion of type I fibers was observed in diaphragm of emphysematous COPD patients compared with controls. Moreover, capillary density, mitochondrial function, and oxidative enzyme activities were enhanced in diaphragm of normal-weight COPD patients compared with controls, reflecting an endurance training-like effect potentially resulting from the increased work of breathing (13). Collectively, diaphragm and peripheral muscles show similar abnormalities with regard to atrophy, proteasome activity, oxidative stress, and regenerative signaling, and these abnormalities may originate systemically. However, differences between intrinsic abnormalities in oxidative phenotype in peripheral vs. diaphragmatic muscle in COPD suggest that local factors, such as activity level, likely participate in the muscle modifications observed in COPD.

ACCELERATORS OF CACHEXIA IN COPD

The fact that many of the impairments in (peripheral) COPD muscle oxidative phenotype seem more pronounced in either cachectic patients or weight loss-susceptible emphysematous patients (53, 79) suggests a relationship between muscle wasting and intrinsic changes in the muscle's oxidative profile. Here we postulate that loss of skeletal muscle oxidative phenotype could potentially accelerate the cachexia process in COPD, as outlined in Fig. 1.

Energy Metabolism

Weight loss occurs when energy expenditure exceeds energy intake. Decreased dietary intake is sometimes present in COPD, in particular during acute exacerbations, and has been related to hypoxia, systemic inflammation, and elevated levels of the appetite-regulating hormone leptin (adjusted for fat mass) (18, 102). However, anorexia is not the primary cause of weight loss in clinically stable disease (15). First, severe COPD patients with a low BMI displayed an increased, not decreased, caloric intake (84). Moreover, although the normal response to (semi)starvation is reducing resting energy expenditure (EE), severe COPD patients often displayed an increased resting EE even when corrected for FFM (51). Collectively, this suggests a hypermetabolic state that may contribute to weight loss if caloric demand is not fully met.

In addition to an increased resting EE, activity-induced EE is increased in COPD that, compared with resting EE, contributes most to elevated total daily EE (93). Increases in activity-induced EE in COPD may be explained by a decreased mechanical efficiency (the chemical conversion of energy to mechanical work) during lower limb exercise (5, 85). Recently, a higher ATP cost of muscle contraction in COPD patients vs. controls was found, which further supports the lower mechanical efficiency hypothesis (58).

Other factors that may well contribute to increases in both resting and activity-induced EE in COPD include impairments in the muscle's oxidative phenotype, increased reliance on glycolytic metabolism, and I-II fiber-type shifting (i.e., metabolic inefficiency). In addition, the oxygen cost of breathing is higher (attributable to airway obstruction and hyperinflation) in COPD, which may also increase EE (5). Moreover, protein turnover is a major determinant of EE (110), and whole body protein turnover has been shown to be increased in COPD, which correlated with increased resting EE and loss of FFM (51, 87).

Summarized, metabolic inefficiency, attributable to impairments in muscle oxidative phenotype, may lead to a decreased mechanical efficiency and increased EE resulting in an energy deficit that subsequently contributes to a wasting phenotype. This is supported by a study showing that mild muscle-specific overexpression of PGC-1α, a key regulator of muscle oxidative capacity that is decreased in COPD muscle, protects against

aging-associated activation of degradation pathways involved in muscle atrophy (109). In this context, it can be speculated that diaphragmatic muscle in COPD may be relatively protected against atrophy because of its enhanced oxidative phenotype.

Oxidative Stress and Inflammation

Oxidative/nitrosative stress and inflammation can be considered as not mutually exclusive processes that can act as potential accelerators of cachexia. Indeed, inflammatory cytokines can induce oxidative stress (80), which is a known mechanism leading to muscle damage and wasting (75), but can also directly induce proteosomal degradation of muscle proteins. This most likely is mediated through activation of the classical NF-κB pathway (56, 81), which has been shown to be activated in skeletal muscle of COPD patients (2).

In addition, we previously showed that TNF-α can induce a shift in MyHC composition toward more fast glycolytic (type II) MyHC proportion (82). Whether or not induced by inflammation, a shift in fiber type composition toward a more glycolytic, type II fiber proportion in COPD may contribute to the process of muscle atrophy. First, as type II fibers are more sensitive to atrophy-inducing stimuli (96), a shift toward a higher proportion of glycolytic fibers may increase the susceptibility of the muscle to inflammation-induced and oxidative stress-induced damage. Moreover, it has been shown that mitochondria from glycolytic type II fibers release significantly more ROS compared with mitochondria from oxidative type I fibers (71). This implies that a shift in fiber-type composition toward a higher proportion of type II fibers may contribute to increased oxidative stress and damage to muscle proteins. Also, loss of muscle oxidative phenotype, whether or not induced by inflammatory cytokines and classical NF-κB activation, can contribute significantly to metabolic inefficiency, which, as described above, may also contribute to wasting. Interestingly, TNF-α potently impaired the oxidative phenotype of cultured myotubes and COPD patients with elevated mRNA levels of muscle TNF-α displayed decreased expression of oxidative genes, impaired PGC-1α/PPAR expression and a cachectic phenotype compared with COPD patients with normal muscle TNF-α levels (82), suggesting a role for TNF-α in loss of muscle oxidative phenotype in COPD.

In light of these observations and the fact that the fiber-type shift (43, 89), abnormalities in muscle oxidative capacity (79, 82), and levels of oxidative stress (101) appear to be aggravated in cachectic and/or emphysematous COPD patients it can be postulated that an impaired muscle oxidative phenotype as well as the presence of inflammation and oxidative stress could be considered as potential accelerators of cachexia in COPD.

THERAPEUTIC INTERVENTIONS

Protein Synthesis and Regeneration

Several studies in COPD demonstrated that therapeutic interventions aimed at increasing muscle mass induce signaling events involved in protein synthesis. Indeed, increases in leg lean mass and muscle fiber size after either training, testosterone supplementation, or electrical stimulation were paralleled by increases in muscle IGF-1 protein and mRNA levels and increased expression of the load-sensitive splice variant of IGF-1:MGF (mechano-growth factor) and activation of the protein synthesis machinery (61, 104, 106). Only one study addressed the muscle anabolic response in cachectic COPD patients upon pulmonary rehabilitation, which on the basis of muscle IGF-I expression levels, appeared present but attenuated compared with noncachectic patients (105). In addition, studies have shown that physical exercise training in COPD patients activates pathways involved in muscle regeneration. Normal-weight COPD patients and controls display similar training-induced increments in peak work rate and muscle expression of MyoD mRNA, MyoD protein, and myogenin mRNA (61, 106), suggesting that COPD patients retain the capacity to induce peripheral muscle adaptations upon training. Interestingly, rehabilitation-induced increases in MyoD mRNA occurred both in cachectic and noncachectic patients, whereas MyoD protein only increased in noncachectic patients (105). Moreover, fiber size increased in both groups, but significantly less in cachectic than in noncachectic patients, suggesting that cachectic COPD patients may only partially retain the capacity for peripheral muscle remodeling in response to rehabilitation/exercise training.

Protein Degradation

Pulmonary rehabilitation decreased muscle atrogin-1 and MuRF1 expression in noncachectic patients, whereas expression levels were increased in skeletal muscle of cachectic patients in response to rehabilitation (105). Also, Lewis et al. (61) demonstrated a small reduction in myostatin mRNA levels upon pulmonary rehabilitation in combination with testosterone in normal-weight COPD patients (61). Furthermore, quadriceps myostatin mRNA and protein expression were reduced in noncachectic and normal-weight patients upon resistance training and pulmonary rehabilitation, respectively, which, interestingly, was not observed in cachectic patients (98, 105).

Collectively, current therapeutic interventions aimed at increasing muscle mass activate protein synthesis and regenerative signaling and decrease myostatin and UPS E3-ligase expression in skeletal muscle of normal-weight and noncachectic patients. These effects were significantly attenuated or even absent or in cachectic patients.

FUTURE PERSPECTIVES

Many studies include COPD patients with advanced disease (GOLD III-IV). However, to adequately investigate the time course and development of muscle atrophy and aberrations in muscle oxidative phenotype in COPD, longitudinal studies in less-severe COPD patients are important. This will increase insights in the pathophysiology of cachexia and will pave the way for developing novel tailored therapeutic strategies. Also, recent data identified that, in addition to activated protein degradation pathways, protein synthesis signaling via Akt is activated in skeletal muscle of cachectic COPD patients. Future research is needed to unravel whether protein synthesis is indeed activated in peripheral muscle of cachectic COPD patients. Additionally, to date, only one study compared pulmonary rehabilitation-induced muscle adaptations in cachectic patients vs. non-cachectic patients (105). These authors reported that rehabilitation-induced muscle adaptations with respect to activation of signaling pathways involved in protein synthesis and regeneration appear attenuated in cachectic patients compared with noncachectic patients. In light of these data, more studies are needed to adequately address whether cachectic patients retain the capacity for peripheral muscle remodeling upon rehabilitation. Furthermore, to shed more light on the

involvement of apoptosis and impaired regeneration in development of muscle atrophy in COPD, more studies are needed to adequately characterize alterations in muscle apoptotic and regenerative pathways in muscle-atrophied and cachectic patients but also in less severe normal-weight and underweight patients.

CONCLUSION

Our understanding of the mechanisms underlying cachexia and subsequent muscle dysfunction in COPD has increased significantly. Importantly, in addition to BMI, determination of FFMI is crucial to adequately detect muscle wasting and diagnose cachexia in COPD. Strikingly, upon adequate stratification based on body composition, there is an apparent relationship between loss of muscle mass and intrinsic muscle abnormalities in COPD. Indeed, cachectic patients display more severe abnormalities in muscle oxidative phenotype, increased oxidative stress, UPS activation, and impaired regenerative signaling compared with noncachectic patients and muscle-wasted patients. Data with regard to these parameters in normal-weight or underweight patients without significant muscle atrophy or cachexia is often lacking. On the basis of available data however, it can be speculated that loss of peripheral muscle oxidative phenotype may act as an accelerator of cachexia in COPD, a process in which inflammation and oxidative stress may play important roles.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: A.H.R., H.R.G., R.C.L., and A.M.S. conception and design of research; A.H.R. drafted manuscript; A.H.R., H.R.G., R.C.L., and A.M.S. edited and revised manuscript; A.H.R., H.R.G., R.C.L., and A.M.S. approved final version of manuscript; H.R.G. prepared figures.

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